upon exposure to an attenuated virus vaccine, did recently yield promising results in melanoma patients (Tel J, et al. Natural human plasmacytoid dendritic cells induce antigen-specific T-cell responses in melanoma patients. Cancer Res. 2013; 73:1063-75). In mice, cross-talk between pDC and XCR1$^+$ cDC can be critical for the induction of optimal, protective, adaptive immunity to viral infections and also to cancer (Nierkens S, et al. Immune adjuvant efficacy of CpG oligonucleotide in cancer treatment is founded specifically upon TLR9 function in plasmacytoid dendritic cells. Cancer Res. 2011; 71:6428-37) (Zhang Y, et al. Genetic vaccines to potentiate the effective CD103+ dendritic cell-mediated cross-priming of antitumor immunity. J Immunol. 2015; 194:5937-47). Recent correlative data in a human clinical trial does support a protective role of the cross-talk between pDC and XCR1$^+$ cDC for cancer immunotherapy (Sluijter B J, et al. Arming the Melanoma Sentinel Lymph Node through Local Administration of CpG-B and GM-CSF: Recruitment and Activation of BDCA3/CD141(+) Dendritic Cells and Enhanced Cross-Presentation. Cancer Immunol Res. 2015; 3:495-505). The rarity and fragility of human XCR1$^+$ cDC is a major limitation to their direct isolation ex vivo for immunotherapy. Hence, methods of obtaining a mixed population of human XCR1$^+$ cDC and pDC from hematopoietic stem cells are of strong interest to advance our basic understanding of their interactions and as a potential source of cells for immunotherapy. A few studies have reported simultaneous in vitro generation of human XCR1+ cDC and pDC from hematopoietic stem cells but with limited yields (Thordardottir et al. Stem cells and development. 2014; Lee et al. J Exp Med. 2015).

SUMMARY OF THE INVENTION

[0004] The present invention relates to methods of obtaining a mixed population of human XCR1$^+$ and plasmacytoid dendritic cells from hematopoietic stem cells, leading to higher yields than reported previously and including an expansion phase of the precursors before their differentiation making this culture system highly versatile. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION

[0005] The present invention relates also to a method of obtaining a mixed population of human XCR1+ and plasmacytoid dendritic cells comprising the steps of i) culturing a population of human hematopoietic stem cells (HSC) or committed hematopoietic precursor cells in the presence of a Notch ligand, and thereafter, ii) isolating human XCR1+ and plasmacytoid dendritic cells from the culture.

[0006] As used herein, the term "classical dendritic cell" or "cDC" has its general meaning in the art and refers to a population of hematopoietic cells with critical roles in immunity, including immune activation in response to pathogen-elicited danger signals and immune tolerance. These cells are characterized by their distinctive morphology and high levels of surface MHC-class II expression. cDC have a high capacity for sensitizing MHC-restricted T cells, and are the only antigen-presenting cells (APCs) that can efficiently activate naïve T-cells.

[0007] As used herein, the term "XCR1" has its general meaning in the art and refers to the XC chemokine receptor 1. An exemplary human amino acid sequence is represented by the NCBI reference sequence NP_001019815.1. XCR1 is also known as GPRS; CCXCR1.

[0008] As used herein, the term "XCR1$^+$ cDC" has its general meaning in the art and refers to a subset of dendritic cells that specifically express the XCR1 chemokine receptor. Human XCR1$^+$ cDC are particularly efficient for cross-presentation. As components of the innate immune system, these cells express intracellular Toll-like receptors 3 and 8, which enable the detection of viral nucleic acids, such as dsRNA and ssRNA motifs respectively. Upon stimulation and subsequent activation through TLR3, these cells uniquely produce large amounts of Type III interferon (e.g., IFN-λ), which are critical pleiotropic anti-viral compounds mediating a wide range of effects. Upon stimulation and subsequent activation through TLR8, these cells can produce interleukin-12 (IL-12), which is a critical cytokine contributing to promote functional polarization of T lymphocytes towards potent antiviral and anti-tumoral functions.

[0009] As used herein, the term "plasmacytoid dendritic cell" or "pDC" has its general meaning in the art and refers to a subtype of circulating dendritic cells found in the blood and peripheral lymphoid organs. These cells express the surface markers CD123, BDCA-2(CD303), BDCA-4 (CD304) and HLA-DR, but do not express CD11c, CD14, CD3, CD20 or CD56, which distinguishes them from cDC, monocytes, T-cells, B cells and NK cells. As components of the innate immune system, these cells express intracellular Toll-like receptors 7 and 9, which enable the detection of viral and bacterial nucleic acids, such as ssRNA or CpG DNA motifs. Upon stimulation and subsequent activation, these cells produce large amounts of Type I interferon (mainly IFN-α and IFN-β) and Type III interferon (e.g., IFN-λ), which are critical pleiotropic anti-viral compounds mediating a wide range of effects.

[0010] As used herein, the term "hematopoietic stem cell" or "HSC" has its general meaning in the art and refers to immature blood precursor cells having the capacity to self-renew and to differentiate into more mature blood cells comprising granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), monocytes (e.g., monocytes, macrophages), lymphocytes (e.g. B- and T cells), and DC. In particular, hematopoietic stem cell are CD34$^+$ cells. The term "CD34$^+$ cells" refers to cells that express at their surface the CD34 marker. Hematopoietic stem cells and in particular CD34$^+$ cells are typically obtained from blood products. A blood product includes a product obtained from the body or an organ of the body containing cells of hematopoietic origin. Such sources include un-fractionated bone marrow, umbilical cord blood, peripheral blood, liver, thymus, lymph and spleen. All of the aforementioned crude or un-fractionated blood products can be enriched for cells having hematopoietic stem cell characteristics in ways known to those of skill in the art.

[0011] As used herein, the term "committed precursor cells" refers to cells which develop from HSC or CD34$^+$ cells but have a more restricted developmental potential. Consequently, these precursor cells (e.g. macrophage dendritic cell precursor, common dendritic cell precursor, or pre-dendritic cell precursor) are more committed to develop into a particular immune cell lineage (e.g macrophages, DC).